extrasynaptic NMDA receptors has beneficial outcomes. Although these studies represent an important contribution to the understanding of the pathophysiology of HD, this disease involves multiple organs, multiple brain regions and changes in numerous neurotransmitter and receptor systems. It is probable that combination drug therapies, accounting for the multiple brain areas affected, the different ways they are affected, and the progression of the symptoms of HD, will provide the most efficacious approach to treatments.

REFERENCES

André, V.M., Cepeda, C., Venegas, A., Gomez, Y., and Levine, M.S. (2006). J. Neurophysiol. *95*, 2108–2119.

Cepeda, C., Wu, N., André, V.M., Cummings, D.M., and Levine, M.S. (2007). Prog. Neurobiol. *81*, 253–271.

Coyle, J.T. (1979). Biol. Psychiatry *14*, 251–276.

Cummings, D.M., André, V.M., Uzgil, B.O., Gee, S.M., Fisher, Y.E., Cepeda, C., and Levine, M.S. (2009). J. Neurosci. *29*, 10371–10386.

Fan, M.M., and Raymond, L.A. (2007). Prog. Neurobiol. *81*, 272–293.

Joshi, P.R., Wu, N.P., André, V.M., Cummings, D.M., Cepeda, C., Joyce, J.A., Carroll, J.B., Leavitt, B.R., Hayden, M.R., Levine, M.S., and Bamford, N.S. (2009). J. Neurosci. *29*, 2414–2427.

Milnerwood, A.J., Gladding, C.M., Pouladi, M.A., Kaufman, A.M., Hines, R.M., Boyd, J.D., Ko, R.W.Y., Vasuta, O.C., Graham, R.K., Hayden, M.R., et al. (2010). Neuron *65*, this issue, 178–190.

Okamoto, S.-I., Pouladi, M.A., Talantova, M., Yao, D., Xia, P., Ehrnhoesfer, D.E., Zaidi, R., Clemente, A., Kaul, M., Graham, R.K., et al. (2009). Nat. Med. *15*, 1407–1413.

Ondo, W.G., Mejia, N.I., and Hunter, C.B. (2007). Parkinsonism Relat. Disord. *13*, 453–454.

Papadia, S., and Hardingham, G.E. (2007). Neuroscientist *6*, 572–579.

Re, D.B., Nafia, I., Melon, C., Shimamoto, K., Kerkerian-Le, G., and Had-Aissouni, L. (2006). Glia *54*, 47–57.

Rosas, H.D., Hevelone, N.D., Zaleta, A.K., Greve, D.N., Salat, D.H., and Fischl, B. (2005). Neurology *65*, 745–747.

Subramaniam, S., Sixt, K.M., Barrow, R., and Snyder, S.H. (2009). Science *324*, 1327–1330.

The Huntington's Disease Collaborative Research Group. (1993). Cell *72*, 971–983.

Tovar, K.R., and Westbrook, G.L. (2002). Neuron *34*, 255–264.

Excitatory Neuromodulator Reduces Dopamine Release, Enhancing Prolactin Secretion

Anthony N. van den Pol[1,*]
[1]Department of Neurosurgery, Yale University, New Haven, CT 06520, USA
*Correspondence: anthony.vandenpol@yale.edu
DOI 10.1016/j.neuron.2010.01.013

Hypothalamic dopamine neurons inhibit pituitary prolactin secretion. In this issue of *Neuron*, Lyons et al. provide evidence for a novel model, whereby the excitatory neuropeptide TRH depolarizes gap-junction-coupled dopamine neurons, leading to a shift in the population pattern of action potentials from phasic burst firing to regular tonic firing, hypothetically reducing dopamine release while increasing total spike number.

Prolactin is a fascinating pituitary hormone that promotes milk synthesis during lactation; its chemical structure is similar to that of growth hormone. Prolactin also enhances maternal behavior, and paternal behavior in some species, modulates immune responses, and is luteotrophic. Prolactin has also been associated with reduced fertility during lactation, thereby enhancing the direction of maternal energy stores toward a new infant rather than toward pregnancy (Freeman et al., 2000). Most of the functions of prolactin, including lactation, are carried out in concert with other hormones that collaborate to achieve a functional response.

Hypothalamic peptides released by axons in the median eminence regulate hormones synthesized and released by the anterior pituitary, or adenohypophysis. In contrast to other pituitary hormones that are controlled by releasing factors, secretion of prolactin is controlled by an inhibiting factor, dopamine. Dopamine is synthesized by a subset of neurons in the hypothalamic arcuate nucleus, sometimes called tuberoinfundibular dopamine (TIDA) neurons, that release the monoamine from axon terminals in the median eminence. Dopamine is then carried by the portal blood system to the pituitary where it activates dopamine G protein coupled D2 receptors that reduce cAMP and attenuate prolactin release, synthesis, and division of prolactin cells. The current model of CNS control of prolactin secretion is based on the view that when dopamine neurons in the arcuate nucleus stop firing, this leads to a loss of dopamine-mediated inhibition of the prolactin-secreting cells, thereby increasing prolactin release.

In a new paper in *Neuron*, Lyons, Horjales-Araujo, and Broberger (Lyons et al., 2010) from the Karolinska Institute in Sweden record from hypothalamic slices, and demonstrate for the first time that arcuate dopamine neurons show phasic bursts of spikes (20 spikes over 4–5 s)

occurring at 20 s intervals. These oscillator neurons show slow 30 mV depolarizations in membrane potential, with the spike burst occurring at the most depolarized state, followed by rapid hyperpolarization, and then a new cycle of depolarization. Current-voltage analysis revealed two characteristic conductances, anomalous inward rectification and a large A-like transient outward rectification, with no apparent I_h current typical of many other pacemaker neurons. That these cells project to the median eminence was confirmed by recording cells labeled by intravenous Evans Blue dye injection.

Interestingly, spike bursts in different dopamine neurons are temporally coupled. Surprisingly, this coupling was not affected by blockade of synaptic communication, but instead appeared to be based on a mechanism of gap-junction-mediated communication between neurons. Gap junction blockers carbenoxolone or glycyrrhetinic acid attenuated the temporal coupling. In the process of identifying the cells by dye-filling followed by immunocytochemistry, the dendritic architecture of the dopamine neurons was revealed. Unlike other arcuate nucleus neurons that have simple, often bipolar dendritic arbors, dopamine neurons had four to five primary dendrites arising from the cell body. Gap junction morphology, size, type, and frequency in TIDA neurons are unknown. However, ultrastructural studies of TIDA neurons found unusual, selective, and frequent apposition between two dopamine dendrites, or between a dendrite and cell body (Piotte et al., 1985). Although gap junctions were not described, these extensive regions of membrane apposition between dopamine neurons would be the most likely site for gap junctions. Small or infrequent gap junctions between neurons may allow some synchronization over minutes to hours, but the fast burst synchronization here is likely mediated by larger and more frequent gap junctions (Rash et al., 2007).

As many dopamine cells are coupled by the electrotonic junctions, one could imagine a model by which depolarizing current could flow additively from multiple cells during slow depolarization, resulting in maximized synchrony of spike bursts. Furthermore, strong gap junction coupling of multiple cells may enhance depolarization of cells that might otherwise show modest depolarization, and thereby may enhance or initiate bursting in individual cells that alone might not burst. Electrotonic coupling in a neuronal population leads to complex network properties whereby the population tends to show all-or-none network bursts; a weak or diffuse input is shunted throughout the network, whereas a synchronous excitatory input generates (in an all-or-none manner) network bursts.

The underlying assumption on which the Lyons paper rests is that bursting increases dopamine release to a much greater level than tonic firing. If this assumption is correct, the maximal concentration of dopamine in the portal blood supply is further enhanced by the gap-junction-mediated synchronous release of many, perhaps most, of the arcuate dopamine neurons. However, a 4–5 s dopamine release into the median eminence at 20 s intervals will probably not activate receptors in the same square wave pattern, given the dilution by the blood and varying distances to prolactin cells in the pituitary. The temporal pattern of dopamine arriving in the pituitary at prolactin cells merits study to determine the functional role of the short-period pulsatile release of dopamine in its activation of the D2 receptors; it is possible that the critical factor here is not the periodicity of receptor activation, but rather that the periodicity of firing maximizes dopamine release.

Thyrotropin-releasing hormone (TRH), the primary hypothalamic neuropeptide that evokes thyroid-stimulating hormone (TSH) release from the pituitary, has also been shown to enhance prolactin release. Since prolactin cells can respond directly to TRH, it has been assumed that TRH acts directly in the pituitary. However, Lyons et al. show that TRH axonal boutons surround arcuate dopamine neurons, and TRH application depolarizes the dopamine neurons, causing them to shift from a phasic bursting pattern to a regular 1.6 Hz tonic pattern. Lyons et al. point out that the spike frequency during the burst (4 Hz) is substantially greater than the tonic firing rate. However, over time, the total number of spikes recorded during tonic firing (32 spikes/20 s) is actually greater than the mean spike frequency associated with bursting behavior (20 spikes/20 s). Based on previous work measuring oxytocin or vasopressin release from the neurohypophysis, dense-core vesicle secretion is greater per spike in bursts than with regular firing. In parallel, studies of the substantia nigra dopamine neurons find burst firing substantially enhances dopamine release, and gap junctions between nigral dopamine neurons occur, but may not be as frequent as in the arcuate nucleus (Overton and Clark, 1997). The working assumption is that TRH, in its depolarizing initiation of tonic firing in TIDA neurons, substantially reduces dopamine release. But as dopamine release was not measured, this remains a missing piece of the puzzle that will profit from experimental confirmation.

Unresolved questions remain. The experiments of Lyons et al. were done on immature rats; since release of prolactin is principally important in adult mothers, it would be nice to confirm the data with mature female rats. In addition, gap junctions are more common in the immature brain, and therefore corroboration of these results in adults will strengthen the model.

Release characteristics of the fast transmitters glutamate and GABA have been studied in great detail, and the wealth of information available benefits chiefly from our ability to investigate release by studying the easily detected electrophysiological response of postsynaptic cells milliseconds later. In contrast, neuromodulators such as dopamine and most neuropeptides activate G protein coupled receptors that show responses in a substantially slower time frame, making it much more difficult to study release by measuring response. Microdialysis, collecting portal blood, or measuring neuromodulators in peripheral blood is done on a timescale of minutes to hours. Alternately, imaging of exocytotic vesicles can be done with agents such as FM1-43, pH-sensitive dyes, or engineered proteins (Gaffield and Betz,2006; Zhu et al., 2009), but at best this gives information about vesicular fusion and exocytosis, which may be difficult to correlate with neuromodulator release. One promising approach to measuring release from dense-core vesicles is the use of expressed recombinant

genes coding for the neuropeptide FMRF and the invertebrate FMRF receptor that gates ion channels, and therefore allows millisecond time resolution in detecting neuropeptide release (Whim and Moss, 2001).

Many if not most neurons contain both fast transmitters such as glutamate and GABA that are released presynaptically, in addition to neuromodulators including dopamine or TRH that are released by axonal boutons, but not necessarily at the synaptic specialization. In the hypothalamus, ultrastructural serial section analysis showed that most axonal boutons have two types of vesicle, the small clear vesicles congregated at the synaptic junction, and dense-core vesicles that are found farther away from the synaptic specialization (Decavel and van den Pol, 1990). Based on immunostaining of catecholamines and vesicular monamine transporters, dopamine has been suggested to be localized in both types of vesicle (Liu et al., 1994; Nirenberg et al.,1995; Henry et al., 1998). Although hundreds of papers have examined the behavioral or physiological response to injection of neuropeptides and amines into the brain, the actual release of these agents from axon terminals, particularly from dense-core vesicles, is an area that merits more attention. We know relatively little about the release characteristics of most monoamines and neuropeptides from axons within the brain. Much of what we know arises from work done on release of oxytocin and vasopressin from the posterior pituitary, as mentioned above. The posterior pituitary presents a good model of peptide release because of the large number of axons that release measurable levels of peptide (Dutton and Dyball, 1977; Dreifuss et al., 1971). But neuropeptide release in the brain is substantively different than that in the posterior pituitary; brain neuropeptides are localized in smaller dense-core granules that are much less abundant than those in the neurohypophysis. And where amino acid transmitters and dopamine can be synthesized and transported into vesicles locally in axonal boutons, neuropeptides are made in the cell body, and peptide synthesis and transport of the dense-core vesicles to axon terminals may take several hours.

Although TRH is shown to depolarize the membrane potential of arcuate dopamine neurons, the question remains as to whether this is the primary transmitter controlling TIDA neurons to evoke release of prolactin. Injections of TRH can lead to prolactin release, in part by a direct action on pituitary prolactin cells; however, the temporal release of TRH and TSH in animals does not show a high correlation with prolactin release (Freeman et al., 2000); furthermore, since TRH is the primary agent regulating TSH release, it would seem unlikely that two pituitary hormones with strikingly different functions would be regulated by a single releasing factor. One possibility is that different TRH neurons release peptide into the median eminence and onto arcuate nucleus neurons. TRH cells are found in the arcuate, paraventricular, dorsomedial, and lateral hypothalamic nuclei, and other areas of the brain. An alternate possibility is that other transmitters and modulators may also depolarize dopamine neurons and could therefore cause the same resultant shift to tonic firing. In addition to glutamate acting at either ionotropic or metabotropic receptors, a number of neuropeptides, including hypocretin, GLP-1, neuromedin B, gastrin releasing peptide, and additional neuromodulators, excite other neurons of the arcuate nucleus (Acuna-Goycolea and van den Pol, 2009), and may therefore also activate the TIDA cells.

These novel data in Lyon's paper open the door on a new perspective of hypothalamic regulation of prolactin release. Rather than the inhibitory dopamine neurons changing from an active state to a silent state, induced by either GABA or one of the many inhibitory modulators of the arcuate nucleus, the new model here posits that a *reduction* in dopamine release instead is evoked by an *excitatory* neuromodulator that depolarizes the membrane potential and increases the general spike frequency over time, but attenuates the critical phasic bursting of the dopamine cells, thereby reducing dopamine release and increasing prolactin secretion.

REFERENCES

Acuna-Goycolea, C., and van den Pol, A.N. (2009). J. Neurosci. *29*, 1503–1515.

Decavel, C., and van den Pol, A.N. (1990). J. Comp. Neurol. *302*, 1019–1037.

Dreifuss, J.J., Kalnins, I., Kelly, J.S., and Ruf, K. (1971). J. Physiol. *215*, 805–817.

Dutton, A., and Dyball, R.E.J. (1977). J. Physiol. *290*, 433–440.

Freeman, M.E., Kanyicska, B., Lerant, A., and Nagy, G. (2000). Physiol. Rev. *80*, 1523–1631.

Gaffield, M.A., and Betz, W.J. (2006). Nat. Protoc. *1*, 2916–2921.

Henry, J.P., Sagné, C., Bedet, C., and Gasnier, B. (1998). Neurochem. Int. *32*, 227–246.

Liu, Y., Schweitzer, E.S., Nirenberg, M.J., Pickel, V.M., Evans, C.J., and Edwards, R.H. (1994). J. Cell Biol. *127*, 1419–1433.

Lyons, D.J., Horjales-Araujo, E., and Broberger, C. (2010). Neuron *65*, this issue, 217–229.

Nirenberg, M.J., Liu, Y., Peter, D., Edwards, R.H., and Pickel, V.M. (1995). Proc. Natl. Acad. Sci. USA *92*, 8773–8777.

Overton, P.G., and Clark, D. (1997). Brain Res. Brain Res. Rev. *25*, 312–334.

Piotte, M., Beaudet, A., Joh, T.H., and Brawer, J.R. (1985). J. Comp. Neurol. *239*, 44–53.

Rash, J.E., Olson, C.O., Pouliot, W.A., Davidson, K.G.V., Yasumura, T., Furman, C.S., Royer, S., Kamasawa, N., Nagy, J.I., and Dudek, F.E. (2007). Neuroscience *149*, 350–371.

Whim, M.D., and Moss, G.W. (2001). Neuron *30*, 37–50.

Zhu, Y., Xu, J., and Heinemann, S.F. (2009). Neuron *61*, 397–411.